Research Article

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Growth, yield, and secondary metabolite responses of three shallot cultivars at different watering intervals

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Abstract: Indonesia is a significant producer of shallots, and the country frequently cultivates three distinct varieties: Bima, Trisula, and Sumenep. Each cultivar exhibits distinct characteristics, specifically when grown under suboptimal conditions. Several studies showed that insufficient watering intervals (WIs) can lead to drought stress, while excessive levels often lead to inundation. To investigate the effects of cultivar and WI on shallot growth and quality, this study employed a split-plot design with main plots and subplots with four repetitions. The main plots consisted of cultivars (Bima, Trisula, and Sumenep), while the subplots comprised WIs (once in 1 day, 2 days, and 3 days). The results showed that there was an effect of interaction between cultivars and WIs on growth (proline [126.15 mg 100 g^{-1}]) and quality (quercetin [3.8739 mg g^{-1}], rutin [0.2080 mg g^{-1}], and kaempferol [1.209 mg g^{-1}]). However, there was no effect on the number of tillers, shoot/root ratio, water content, number of tubers, tuber weight, and total flavonoid.

Keywords: drought conditions, field capacity, nutritional compounds, quality attributes, water stress

1 Introduction

Shallot (*Allium ascalonicum* L.) is a highly popular horticultural product with several benefits. It can be consumed raw or used as a seasoning for dishes due to their distinctive aroma. Furthermore, shallot can be processed into products, such as pasta, serving as a savory flavor enhancer in food. The plant is also rich in nutrients, such as carbohydrates, vitamins, calcium, and other essential minerals. Several studies reported that it has gained significant attention in the biopharmaceutical industry due to the potential as antimicrobial, anti-diabetic, anti-hypertensive, antioxidant, and analgesic. Various secondary metabolites, including antioxidants, total chlorophyll, and total flavonoids (such as quercetin, flavonol glycosides, kaempferol, and others), are essential components in plants [1–6]. Quercetin, in particular, has been reported to be beneficial to heart health, kidney, liver, and hyperglycemia.

The quality of shallot is highly susceptible to the influence of environmental factors, and climate change can lead to a decrease in water availability due to extended dry seasons and low rainfall. These conditions can cause drought stress, which affected various parameters of plant growth and yield. Moreover, drought stress was reported to reduce the yield of the Bhima Kiran onion cultivar by 65% [7]. Previous studies reported that deficit irrigation at a rate of 20% of crop water demand with a 4-day interval throughout the growing season is advantageous for maximizing onion yield and improving water productivity [8]. Plant growth in drought conditions is often directed towards the roots, thereby facilitating the absorption of water from deeper soil layers [9]. Drought stress can also induce the appearance of proline, which is an essential protein amino acid. Proline often accumulates under drought stress, but its content can decrease rapidly after rewatering [10]. Several studies also revealed that it had the ability to maintain turgor [11]. Another response of plants to drought stress is an increase in biochemical and yield traits of onion. Water deficit influenced bulk weight, diameter, lipid peroxidation, and total soluble solids in onion [7,12].

Each cultivar has different morphological, physiological, and biochemical responses with unique effects on metabolite content [13–18] and the activity of enzymes during the formation of secondary metabolites. This is

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because secondary metabolites are affected by the response of plant genetic traits that are sensitive to stress [19,20]. Furthermore, several studies have been carried out to determine the effect of cultivar type on plant growth, yield, and metabolite content. It is believed to be the biggest factor affecting shallot's secondary metabolites, followed by planting time and cultivation methods [21]. Shallot cultivar PRO-6 contained higher quercetin, kaempferol, and total flavonoid compared to Punjab White, Punjab Naroya, and other commercial variants [22]. A study was conducted on three types of shallot cultivars given different inundation levels. The results showed that the Sumenep samples had better growth and yield compared to the Bima and Kuning variants [23].

The interaction effect of cultivar and WI on shallot plants has been extensively explored. A previous study showed that the association between cultivar and drought stress affected the content of carotenoids in the carrot [24]. Water has a considerable effect on the root's ability to absorb nutrients. A persistent lack of water can result in stunted growth and even death. Therefore, further research is needed regarding the appropriate volume of watering for the shallot. Based on previous findings, only a few studies have explored the effect of WIs on local Indonesian shallot varieties, especially Bima, Trisula, and Sumenep. Therefore, the aim of this study is to determine the interaction and effect of cultivars and different WIs on shallot growth, yield, and quality. The results are expected to be a reference for farmers and the industry in determining the right cultivar and WI to obtain optimal yields.

2 Materials and methods

2.1 Experimental design and treatments

Planting was carried out at the Controlled Culture Laboratory, Faculty of Agriculture, Universitas Padjadjaran, Indonesia, with coordinate of 6°55′12.1″S 107°46′21.6″E and an altitude of ±730 m above sea level. The experimental design used was a split-plot design consisting of main plots, namely, cultivars (Bima, Trisula, and Sumenep) and subplots, including WIs (1, 2, and 3 days) with four replications. Bulbs were collected from Vegetable Crops Research Institute, Lembang, Indonesia. Each treatment consisted of six polybags (two polybags for proline analysis and four polybags for another analysis (number of tillers, number of tubers, tuber weight, shoot/root ratio, tuber water content, drought sensitivity index; DSI, quercetin, rutin, kaempferol, and total flavonoid) with one plant in one polybag (15 × 15 cm). The total number of plants was 216, and the planting media used was 3 kg (mixed from soil, compost, and husk charcoal [1:1:1]) (Figure 1).

The maintenance carried out was watering, replanting, weeding, loosening of soil, fertilization, as well as controlling pests and plant diseases. Table 1 presents the meteorological data (air temperature and relative humidity) recorded from October to December 2020, which provides valuable insight into seasonal variations and environmental conditions during this time period. The meteorological data were measured in the field experiment using thermo-hygrometer. The watering of the shallot plants was based on the treatment. The volume of water given to the treatment was adjusted to the field capacity of 200 mL for one watering, which was carried out from 2 weeks after planting (WAP) until harvest (15–17 WAP). Replanting was performed at 1 WAP to replace plants that were not growing. Basic fertilization was carried out a day before planting, namely, urea (1 g $polybag^{-1}$), ZA (2.5 g $polybag^{-1}$), SP-36 (1.5 g $polybag^{-1}$), and KCl (1 g $polybag^{-1}$), respectively. Meanwhile, supplementary fertilization was done 15 and 35 days after planting.

Figure 1: Shallot plants in various treatments (Field shaded with UV plastic).

Table 1: Meteorological data (air temperature and relative humidity) collected from October to December 2020

No.	Month	Air temperature (°C)	Relative humidity (%)
1	October	27.6	62.4
2	November	26.0	71.5
3	December	26.0	70.6

Basic and supplementary fertilization are done at the same dosage. The fertilizer mixture was then stirred and spread over the soil in the polybags.

2.2 Growth and yield determination

Shallot was harvested after they were matured, usually at the age of 15–17 WAP [25]. This period was characterized by the softness of 60% of the stem neck being lodged and the yellow coloration of the leaves. Harvesting was carried out in dry soil conditions and sunny weather to prevent tuber rot disease in the warehouse. The harvested shallot was then tied to the stem to facilitate handling. The tuber was dried in the sun for 1–2 weeks under direct sunlight. Subsequently, laboratory analysis was performed at the Horticulture Laboratory

2.2.1 Number of tillers

The number of tillers per clump calculation was carried out from the age of 1–7 WAP. The value was obtained by counting all tillers of the sample plants, and the average was calculated for each plot.

2.2.2 Number of tubers

The number of tubers was the average number of sample plants from the plots. This observation was carried out during the harvest of the plants.

2.2.3 Tuber weight

The tuber weight was the average weight of tuber per plant clump. The sample consisted of three plants in each treatment and replication. The weight was calculated by weighing the tuber after harvesting.

2.2.4 Shoot/root ratio

The ratio between the shoot dry weight and root dry weight was calculated (equation (1)). For this, the plants were harvested at 7 WAP, and the shoot separated from the roots (were washed to remove from the soil). The fresh material was placed in paper bags and dried in a forced-air oven (Memmert Schutzart DIN 40050-IP 20, Schwabach, Germany) for 3 days at 105°C to quantify the shoot and root dry weights. Dry weight was obtained using a digital balance.

$$\text{Shoot/root ratio} = \frac{\text{Shoot dry weight}}{\text{Root dry weight}}. \tag{1}$$

2.2.5 Proline

Also, in the harvest (7 WAP), proline measurement was carried out based on the method proposed by Bates et al. [26]. A total of 5 g fresh leaf samples was crushed using a mortar, followed by the addition of 10 mL of 3% sulfosalicylic acid and homogenization using a vortex. The homogenized sample was then placed in boiling water (100°C) for 10 min. Subsequently, a total of 1 mL filtrate was reacted with 1 mL ninhydrin acid and 1 mL glacial acetic acid in a test tube for 40 min at 100°C. The reaction solution was then placed in cold water for 1 min, followed by extraction with 3 mL of toluene and measurement of absorbance using a spectrophotometer (Shimadzu UV mini-1240, Tokyo, Japan) at a 520 nm wavelength. The analysis of each sample was repeated three times, and the proline concentration was calculated by including it in the standard curve. The proline was calculated according to equation (2).

$$\text{Proline(mg/100 g)} = \frac{C \times V}{m} \times 100, \tag{2}$$

where C is the concentration of the proline obtained from calibration curve in mg mL^{-1}, V is the volume of extract in mL, and m is the mass of extract in g.

2.2.6 Tuber water content

Water content was measured based on the gravimetric method [27–30]. A total of 15 g shallot was weighed in a cup using aluminum foil with known weight, followed by drying in an oven at 105°C for 3 h. The samples were cooled in a desiccator for 15 min and weighed. Subsequently, they were placed back in the oven for 2 h after being cooled and weighed. This treatment was repeated until a constant weight was obtained.

2.2.7 DSI

DSI was calculated to determine the yield reduction caused for each treatment compared to the control. Cultivars with low (<1) and high (>1) DSI values were categorized as drought-tolerant and drought-sensitive, respectively [31]. The formula used to calculate DSI is

$$\mathrm{DSI} = \frac{\left(1 - \frac{Y}{\mathrm{Yp}}\right)}{\left(1 - \frac{X}{\mathrm{Xp}}\right)}, \tag{3}$$

where Y is the average weight of shallot bulbs in each cultivar (Bima, Trisula, and Sumenep) under drought stress (WIs of once in 2 and 3 days), Yp is the average weight of shallot bulbs in each cultivar (Bima, Trisula, and Sumenep) without drought stress (WI of once in 1 day), X is the average weight of shallot bulbs across all cultivars under drought stress (WIs of once in 2 and 3 days), and Xp is the average weight of shallot bulbs across all cultivars without drought stress (WI of once in 1 day).

2.3 Quality determination

2.3.1 Extract preparation

The sample was part of shallot tuber (tubers and tuber skins) that were harvested and cleaned to remove soil. The tuber was then sliced, dried in an oven at 50°C for 18 h, mashed with mortar, and referred to as dried samples. [32]. A total of 0.5 g of the dry sample was then measured and placed in a vial, followed by the addition of 10 mL of ethanol (96%). The filtrate was then sonicated (Baku BK-2000, Guangzhou, China) for 30 min at room temperature. The dried sample was used for determining of flavonoids in shallot, such as quercetin, rutin, and kaempferol, and total flavonoid.

2.3.2 Quercetin, rutin, and kaempferol

A total of 5 mL sulfuric acid was added to the filtrate in a fume hood, followed by sonication at 80°C for 45 min, and the addition of ethanol. Furthermore, 10 mL filtrate was centrifuged for 10 min, and 1 mL was analyzed in HPLC (Shimadzu, LC 20AT Prominence, Tokyo, Japan). The measurement of quercetin, rutin, and kaempferol was then carried out using their standards, and the results were expressed as mg g^{-1} [33,34].

2.3.3 Total flavonoid

The total flavonoid testing procedure was based on the method proposed by Sytar et al. [35] and Kusumiyati et al. [36]. A total of 0.5 mL filtrate was added with 2 mL of methanol, 0.1 mL of $AlCl_3$ 10%, 0.1 mL of sodium acetate 1 M, and 2.3 mL of water. The mixture was then incubated for 30 min, and the absorbance was measured at 432 nm using a spectrophotometer. The total flavonoid is calculated according to equation (4).

$$\text{Total flavonoid(mg QE/g dw)} = \frac{C \times V}{m}, \tag{4}$$

Where C is the concentration of gallic acid obtained from calibration curve in mg mL^{-1}, V is the volume of extract in mL, and m is the mass of extract in g.

2.4 Data analysis

The data were analyzed statistically using split plot analysis of variance (ANOVA). In addition, when the test results are substantially different, Tukey test is conducted at a 5% significance level. Data analysis was done using SPSS 16 software and Microsoft Excel 2013.

3 Results and discussion

The statistical analysis was performed with the SPSS 16 software and Microsoft Excel 2013. ANOVA is used to determine whether there is a significant difference between the groups under study by using variance (Table 2). The Tukey test compares the variables after collecting data. Observations of shoot/root ratio and proline were then performed at 7 WAP. The number of tillers was checked at several

Table 2: Probability of growth, yield, and metabolite responses of three shallot cultivars at different WIs

Variables	Cultivar	WI	Cultivar*WI	CV (%)
Number of tillers				
1 WAP	0.014*	0.973 ns	0.671 ns	25
2 WAP	0.050*	0.405 ns	0.559 ns	19
3 WAP	0.037*	0.293 ns	0.727 ns	19
4 WAP	0.045*	0.548 ns	0.481 ns	23
5 WAP	0.018*	0.184 ns	0.361 ns	21
6 WAP	0.045*	0.129 ns	0.598 ns	18
7 WAP	0.050*	0.282 ns	0.167 ns	18
Shoot/root ratio	2.677 ns	13.204*	2.179 ns	33
Proline	0.000*	0.000*	0.000*	47
Tuber water content	0.000*	0.007*	0.242 ns	6
Number of tubers	0.413 ns	0.000*	0.070 ns	22
Tuber weight	0.004*	0.000*	0.126 ns	35
Quercetin	0.000*	0.008*	0.000*	76
Rutin	0.000*	0.001*	0.000*	46
Kaempferol	0.000*	0.108 ns	0.001*	57
Flavonoid	0.000*	0.000*	0,085 ns	57

ns, * – not significant and significant at $p \le 0.05$ by F-test.

frequencies, namely, 1, 2, 3, 4, 5, 6, and 7 WAP. Meanwhile, the number of tubers, tuber weight, quercetin, rutin, kaempferol, total flavonoid, and water content were measured after the harvest.

3.1 Number of tillers

The statistical analysis results showed that there was no effect of interaction of shallot cultivars and the WI on the number of tillers (Table 2). The single factor of each cultivar had a significant effect, while the WI did not affect this parameter. Furthermore, Table 3 shows that the Sumenep cultivar was significantly different from Trisula but was not different from Bima at the age of 1 WAP. The number of tillers at 2–7 WAP in Bima and Sumenep was not significantly different but varied from Trisula. The treatment of the WI did not significantly differ based on the measured parameter, as shown in Table 3. This is in line with the research by Nur et al. [37] who reported that the number of tillers of Bima and Sumenep varieties did not differ significantly at 4 and 6 WAP. It is suspected that genetic factors have a substantial impact on the number of tillers [37,38]. Bima has the potential for 7–12 tillers, while Sumenep for 7–14 tillers The number of tillers was more affected by the seeds used as seedlings compared to water availability. Fibrianty et al. [39] reported that watering volumes did not have significant effect on the number of tillers in shallot.

3.2 Shoot/root ratio

The statistical analysis results showed that there was no impact of interaction between shallot cultivars and WI on the shoot/root ratio (Table 2). The single-factor treatment of cultivar and WI affected this parameter, as shown in Table 4. The shoot/root ratio was the ratio between the crown and roots of the plant. Furthermore, Sumenep produced the highest value compared to other cultivars but did not differ from the Bima variant. The treatment with a WI of once in 1 day gave the highest ratio, followed by once in 2 days and 3 days.

A low shoot/root ratio indicated that plant growth occurred more in roots. Photosynthate directed to this organ was a plant mechanism in expanding and deepening the root system, thereby increasing the water-absorbing ability [9]. Table 4 also shows that there was a decrease in the shoot/root ratio for the WI treatment. This reduction was the plant's effort to maintain water availability for growth and development under drought stress [40]. The accumulation of salts in the topsoil and root zone as a result of water deficiency or drought can substantially reduce onion growth and yield [41]. Soil moisture is depleted as a result of

Table 4: Effect of shallot cultivars and WI on shoot/root ratio

Treatment	Shoot/root ratio
Main plots (cultivar)	
Bima	2.56 ± 1.03ab
Trisula	2.17 ± 0.99a
Sumenep	2.78 ± 0.75b
Subplots (WI)	
Once in 1 day	3.35 ± 0.52c
Once in 2 days	2.32 ± 0.97b
Once in 3 days	1.66 ± 1.00a

Notes: Treatment average numbers followed by the same letter indicate not significantly different according to Tukey's 5% further test. Values represent mean: ± standard deviation.

Table 3: Effect of shallot cultivars and WI on number of tillers

Treatment	Number of tillers						
	1 WAP	2 WAP	3 WAP	4 WAP	5 WAP	6 WAP	7 WAP
Main plots (cultivars)							
Bima	2.12 ± 0.39ab	3.96 ± 0.83a	4.46 ± 0.84a	5.06 ± 1.50a	5.64 ± 1.20a	6.14 ± 1.15a	5.34 ± 1.33a
Trisula	2.25 ± 0.46b	5.08 ± 0.43b	5.52 ± 0.68b	6.64 ± 0.78b	7.24 ± 0.71b	7.19 ± 0.71b	7.07 ± 0.77b
Sumenep	1.78 ± 0.55a	4.52 ± 0.99a	4.83 ± 1.05ab	5.68 ± 1.26a	5.86 ± 1.29a	5.99 ± 0.96a	6.22 ± 1.28a
Subplots (WI)							
Once in 1 day	2.08 ± 0.44a	4.56 ± 0.95a	4.99 ± 1.08a	5.90 ± 1.78a	6.15 ± 1.32a	6.36 ± 1.14a	6.17 ± 1.07a
Once in 2 days	2.00 ± 0.54a	4.29 ± 0.79a	4.57 ± 0.79a	5.43 ± 1.05a	5.68 ± 1.12a	6.17 ± 1.02a	5.99 ± 1.13a
Once in 3 days	2.08 ± 0.52a	4.50 ± 0.78a	4.96 ± 0.88a	5.90 ± 1.47a	6.71 ± 1.47a	6.33 ± 1.15a	6.22 ± 1.39a

Notes: Treatment average numbers followed by the same letter indicate not significantly different according to Tukey's 5% further test; Values represent mean ± standard deviation.

root absorption, resulting in decreased physiological activities, which in turn affects the root development [42].

3.3 Proline

The statistical analysis results showed that there was an effect of interaction between shallot cultivars and WI on proline, as shown in Tables 2 and 5. A WI of once a day had no significant effect on the three cultivars, but there was a significant difference in the once in 2 days and 3 days treatments. This was because the use of the once-a-day treatment helped to prevent stress. Consequently, the proline produced did not show a significant difference in the three cultivars. A significant effect was found in the once in 2 days and 3 days, indicating that the plants experienced stress and responded by increasing the levels of the amino acid.

Proline is an amino acid that acted as one of the most common compatible osmolytes in plants under drought stress conditions. The results showed that its levels increased with the less frequent WIs in each cultivar. This indicated that proline production increased in a stressed environment. Furthermore, its production was a mechanism to reduce oxidative damage. Proline also played three main roles in plants under stress, namely, as a molecular messenger, antioxidative defense molecule, and signaling molecule. Its role as a molecular messenger can regulate protein integrity and increase the activity of different enzymes. Previous studies reported that it had a role in reducing the effects of Reactive Oxygen Species (ROS) such as OH^-, O_2^-, H_2O_2, and others as an antioxidant. ROS can cause oxidative damage in plants through the peroxidation of lipid membrane components and direct interaction with various macromolecules.

The highest proline value was found in Trisula, which was watered once in 3 days at 126.15 mg/100 g fresh sample, compared to Bima and Sumenep with once in 3 days treatment. This showed that Trisula was the best cultivar for overcoming drought stress compared to others. High proline indicated that a plant was tolerant to stress and can adapt to a stressed environment. Drought-stress-tolerant species have higher proline and antioxidant content compared to drought-stress-sensitive species [43]. Several studies reported that proline was an indicator in the selection of stress-tolerant cultivars, such as peanut [44], shallot [45], soybean [46], mung bean [47], and potato [48].

3.4 DSI

The results of the variance analysis are presented in Figure 2. The DSI value of the Bima, Trisula, and Sumenep cultivars was 0.8592, 0.8455, and 1.0078, respectively. Based on the DSI category, it can be concluded that they were drought stress tolerant (DSI value <1). This category can be considered when selecting shallot cultivars planted in drought stress [31]. Bima, Trisula, and Sumenep are cultivars that are adaptive and tolerant to drought stress [49,50].

3.5 Water content, number, and weight of tuber

The statistical analysis results showed that there was no effect of interaction between shallot cultivars and WI on the water content, as well as the number and weight of

Table 5: Effect of interaction between shallot cultivars and WI on proline

Cultivar	Proline (mg/100 g)		
	Once in 1 day	Once in 2 days	Once in 3 days
Bima	37.85 ± 3.01a A	66.71 ± 5.36b AB	83.93 ± 23.32c A
Trisula	51.79 ± 3.24a A	67.89 ± 13.44b B	126.15 ± 14.24c B
Sumenep	28.69 ± 4.00a A	51.87 ± 4.31b A	68.31 ± 10.28c A

Notes: Numbers followed by the same letter are not significantly different according to Tukey's further test at the 5% level. Lowercase letters read horizontal direction (rows) and capital letters read vertical direction (columns). Values represent mean ± standard deviation.

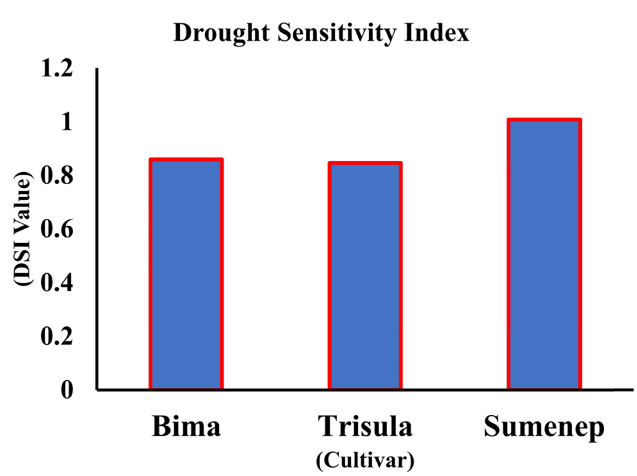

Figure 2: DSI values of some shallot cultivars.

tubers (Table 2). The cultivar and WI treatments showed a single-factor effect on these parameters, as shown in Table 6. The cultivar-type treatment had a significantly different effect on the water content of tuber. Bima had the highest tuber water content compared to Trisula and Sumenep. Meanwhile, Sumenep produced the lowest tuber water content of 62.02%. It was usually used as fried shallot and expected to have a low water level to ensure the texture remains crispy. The water content is an indicator of fried shallot quality because it can affect the texture, and excessive levels prevented the samples from being crispy. This parameter can also affect the shelf life of shallot. Tuber with high water content was more susceptible to disease attacks during the post-harvest period. The character of shallot is mostly impacted by genetics and almost marginally by the environment [51]. The level in shallot ranged from 80.03 to 87.54% and an excessively low level can reduce product weight, while high content caused the occurrence of microbes [52].

The treatment of WI once in 1 day produced the highest water content compared to others. However, it was not significantly different from the once in 2 days. This occurred because the WI affected the amount of water that can be absorbed in the soil by plants. The higher the amount of water available in the soil, the higher the quantity absorbed by plants for growth and development [53]. The moisture absorbed was stored in the tuber and indicated by the tuber water content.

The WI showed an effect on the number of tubers. The results of the Tukey test at 5% level are presented in Table 6. Bima was not significantly different from Trisula and Sumenep. This was because the number of shallot tubers of Bima, Trisula, and Sumenep was the same, namely 5–8 tubers.

The treatment with watering once in 1 day had the highest number of tuber compared to others. This was because water was one of the factors that affected plant yield. Watering with increasingly infrequent intervals caused disrupted fruit formation and enlargement, which reduced fruit size and yield. The once in 1 day treatment increased water availability in the root zone, thereby leading to high shallot production [54].

The cultivar and WI had an effect on tuber weight, as shown in Table 6. Furthermore, Trisula had the highest tuber weight of 39.29 g compared to others, but not significantly different from Bima. The results showed that Sumenep produced the lowest of 26.80 g. The WI treatment had a significantly different effect on tuber weight. The administration of water once in 1 day was significantly different from once in 2 days and 3 days. The once in 2 days was not significantly different from the once in 3 days treatment. The once in 1 day interval gave the highest results, namely, 37.51 g. Several studies have been carried out to determine the effect of drought stress on shallot yield. Water deficit caused a significant decrease in tuber weight and size [55,56]. Stressed plants can give lower yields compared to those that were unstressed as a form of morphological and physiological changes in cultivars susceptible to stress, leading to smaller cell sizes [57].

3.6 Quercetin

The statistical analysis results showed that there was an effect of interaction between shallot cultivars and the WI on quercetin, as shown in Tables 2 and 7. Several studies showed that secondary metabolites can increase due to water stress [58,59]. This was consistent with the results of Bima and Sumenep but different in Trisula. Meanwhile, Bima and Sumenep experienced an increase in quercetin and a decrease in the WI. Trisula experienced a decrease in quercetin at the watering of once in 2 days but there was a slight increase in quercetin at once in 3 days. This indicated that quercetin levels can decrease due to increased

Table 6: Effect of shallot cultivars and WI on the tuber water content, number of tubers, and tuber weight

Treatment	Tuber water content (%)	Number of tubers	Tuber weight (g)
Main plots (cultivar)			
Bima	69.78 ± 1.91c	8.33 ± 2.27a	38.23 ± 15.75b
Trisula	66.47 ± 2.54b	8.06 ± 1.12a	39.29 ± 10.74b
Sumenep	62.02 ± 1.85a	7.38 ± 1.75a	26.80 ± 9.80a
Subplots (WI)			
Once in 1 day	66.54 ± 4.09b	8.14 ± 1.42b	37.51 ± 13.81b
Once in 2 days	65.86 ± 4.05ab	6.86 ± 1.45a	28.06 ± 7.26a
Once in 3 days	64.48 ± 3.40a	6.75 ± 1.19a	23.36 ± 9.14a

Notes: Treatment average numbers followed by the same letter indicate not significantly different according to Tukey's 5% further test. Values represent mean ± standard deviation.

Table 7: Effect of interaction between shallot cultivars and WI on quercetin

Cultivar	Quercetin (mg g^{-1})		
	Once in 1 day	Once in 2 days	Once in 3 days
Bima	1.4207 ± 0.40a B	1.6674 ± 0.44ab B	1.7168 ± 0.35b B
Trisula	3.8739 ± 0.98b C	2.4389 ± 0.82a C	2.8620 ± 0.58a C
Sumenep	0.6555 ± 0.28a A	0.7097 ± 0.12a A	0.7234 ± 0.09a A

Notes: Numbers followed by the same letter are not significantly different according to Tukey's further test at the 5% level. Lowercase letters read horizontal direction (rows) and capital letters read vertical direction (columns). Values represent mean ± standard deviation.

drought stress. Cultivar was the primary factor contributing to the varying levels of secondary metabolites [60].

3.7 Rutin

The statistical analysis results showed that there was an effect of interaction between shallot cultivars and the WI on rutin, as shown in Tables 2 and 8. Bima was not significantly different from Trisula on rutin at WIs of once in a day, but significantly different from Sumenep.

Rutin in Bima and Sumenep watered once in 1 day was not significantly different from those treated once in 2 days. However, it was significantly different from samples watered once in 3 days. This finding is consistent with Toscano et al. [61] that plant secondary metabolites increased under stressful conditions. There was a decrease in Trisula, which could be due to genetic factors. Furthermore, stressed conditions can be associated with the provision of little water, which caused drought. Trisula watered once in 1 day was significantly different from those watered once in 2 days and 3 days.

3.8 Kaempferol

Based on the statistical analysis results, there was an effect of interaction between shallot cultivars and the WI on kaempferol, as shown in Tables 2 and 9. The highest value was obtained in the treatment of Trisula with a WI of once in 1 day. It was significantly different from the samples watered once in 2 days and 3 days. A previous study revealed that an increase in the kaempferol level was a defensive effort under a stressed environment [62].

In Trisula watered once in 1 day, 2 days, and 3 days, there was a significantly different decrease in kaempferol. This occurred due to the effect of the enzymes flavanone 3-hydroxylase (F3H) and FLS, which played a role in converting Dyhydroflavonol into Dihydrokaempferol and Dihydromyricetin [63]. The role in catalyzing the conversion of dihydroflavonols to flavonols, FLS1 also facilitates the oxidation of 2S-flavanone (naringenin) to both enantiomers of dihydrokaempferol, a function typically attributed to F3H [64]. In addition, when the functioning enzymes FNS I and F3H were exposed to dihydrokaempferol as a substrate, it was shown that both FNS I and FNS I/F2H enzymes successfully catalyzed the conversion of dihydrokaempferol into kaempferol [64]. Moreover, the amount of carbon available in plants that were used for total flavonoid biosynthesis can cause a decrease in kaempferol (including total flavonoids). Total flavonoid biosynthesis can increase due to highly available carbon levels and enzyme activity [65].

Table 8: Effect of interaction between shallot cultivars and WI on rutin

Cultivar	Rutin (mg g^{-1})		
	Once in 1 day	Once in 2 days	Once in 3 days
Bima	0.1670 ± 0.066a B	0.1756 ± 0.084a B	0.2080 ±0.041b B
Trisula	0.2049 ± 0.050b B	0.1673 ± 0.072a B	0.1465 ±0.046a B
Sumenep	0.0654 ± 0.050a A	0.0655 ± 0.034a A	0.0669 ±0.008b A

Notes: Numbers followed by the same letter are not significantly different according to Tukey's further test at the 5% level. Lowercase letters read horizontal direction (rows) and capital letters read vertical direction (columns). Values represent mean ± standard deviation.

Table 9: Effect of interaction between shallot cultivars and WI on kaempferol

Cultivar	Kaempferol (mg g^{-1})		
	Once in 1 day	Once in 2 days	Once in 3 days
Bima	0.0866 ± 0.041a B	0.1028 ± 0.050a B	0.1037 ± 0.028a B
Trisula	1.209 ± 0.050b C	0.1981 ± 0.049a C	0.1830 ± 0.032a C
Sumenep	0.0476 ± 0.019a A	0.0621 ± 0.008b A	0.0475 ± 0.013a A

Notes: Numbers followed by the same letter are not significantly different according to Tukey's further test at the 5% level. Lowercase letters read horizontal direction (rows) and capital letters read vertical direction (columns). Values represent mean ± standard deviation.

3.9 Total flavonoid

The statistical analysis results showed that there was no effect of interaction between shallot cultivars and WI on total flavonoids, as shown in Tables 2 and 10. Cultivar treatment and WI affected the levels of total flavonoids.

Cultivar affected the total flavonoid observation parameters, where Trisula showed significantly different results from Bima and Sumenep. Furthermore, Trisula had the highest total flavonoid of 334.73 mg g^{-1}, while Sumenep had the lowest of 95.07 mg g^{-1}. High levels indicated that plants have a better ability to reduce the effect of environmental stress [66]. Based on the results of total flavonoid measurements, Trisula was a cultivar that adapted better to drought stress's effects compared to others.

The treatment of WIs showed a significantly different effect on total flavonoids, where the administration of water once in 1 day varied from others. Treatment with watering once in 2 days was not significantly different from once in 3 days. The once in 1 day watering had the highest total flavonoid at 227.94 mg g^{-1}. Meanwhile, the once in 2 days and 3 days of treatments were not significantly different. The results showed that the higher the stress level given, the lower the total flavonoid in the plant. This occurred because drought stress had a detrimental effect on total flavonoids. The levels often increased in plants that experienced stress, but some studies found a decrease. Alizadeh Yeloojeh et al. [67] stated that total flavonoid content depended on the species, plant parts, and the level of drought.

The decrease in total flavonoids was also caused by the inactivation of enzymes involved in their biosynthesis. One of the enzymes with an important role was F3H [68], which reduced ROS levels in plants under stress. A previous study revealed that drought stress caused a decrease in F3H enzyme activity in *Achillea pachycephala* plants, leading to a decrease in total flavonoids [63]. Liu et al. [69] reported that different gene expression levels caused varying levels as a plant mechanism to survive in a stressed environment.

Table 10: Effect of shallot cultivars and WI on total flavonoid

Treatment	Total flavonoid (mg QE g^{-1} dw)
Main plots (cultivar)	
Bima	221.78 ± 0.76b
Trisula	334.73 ± 1.09c
Sumenep	95.07 ± 0.30a
Subplots (WI)	
Once in 1 day	227.94 ± 1.51b
Once in 2 days	180.89 ± 1.26a
Once in 3 days	178.08 ± 0.80a

Notes: Treatment average numbers followed by the same letter indicate not significantly different according to Tukey's 5% further test. Values represent mean ± standard deviation.

4 Conclusion

Bima, Trisula, and Sumenep cultivars with different WIs had significant impact on growth (proline) and quality (quercetin, rutin, and kaempferol) parameters of shallot. Furthermore, the interaction of Trisula and watering once in 1 day gave the best results on growth, yield, and metabolite responses. The best interval for Bima, Trisula, and Sumenep on growth and yield was once in 1 day, while once in 3 days treatment can increase proline content. WI of once in 1 day gave the best results for shoot/root ratio (3.35), tuber water content (66.54%), number of tubers (8.14), tuber weight (37.51 g), and total flavonoid (227.94 mg g^{-1}).

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